Eubacterium plautii (Séguin 1928) comb. nov.

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The type strain (ATCC 29863 = VPI 0310) of *Fusobacterium plautii* has a multilayered cell envelope structure consisting of a barely visible cell membrane, a dense intermediate layer, and a single-layered outer membrane. The cells do not have flagella and are not motile in hanging drop preparations. The results of this and earlier investigations indicate that despite having a negative Gram reaction, this strain is a gram-positive bacterium with a gram-variable cell wall structure. Since the type strain of *F. plautii* fits the generic description of *Eubacterium*, the species is transferred to this genus as *Eubacterium plautii* (Séguin) comb. nov.

In 1907, Plaut (17) published a morphological description of motile fusiform bacteria that were isolated from ulceromembranous stomatitis in humans. He described the following two main forms: large fusiform rods with barely visible appendages all over the cell surface and a group of smaller, curved rods with a few, clearly visible flagella attached to either the ends or the concave sides of the cells. The second form was morphologically identical to "*Spirillum sputigenum*" described earlier by Miller (14) (names in quotation marks are not on the Approved Lists of Bacterial Names [20], have not been validly published since 1 January 1980, and therefore have no standing in nomenclature). The name *Fusobacterium plauti* (sic) was introduced by Séguin (19), who succeeded in isolating the motile fusiform organism in pure culture. In smears from the cultures Séguin recognized the different morphological forms described by Plaut and concluded that the large motile organism and "*S. sputigenum*" of Miller represented different forms of the same anaerobic vibrio. The description of *Fusobacterium plauti* (sic) Séguin in *Bergey's Manual of Determinative Bacteriology*, 8th ed., is based mainly on the four known strains (VPI 0310, VPI 0311, VPI 0312, and VPI 4145) isolated by Prévot from cultures of *Entamoeba histolytica* (15).

During a study of the fatty acids of different fusobacteria, we found that the patterns of the *Fusobacterium plautii* strains differed markedly from those of the strains of other *Fusobacterium* species (11). 3-Hydroxy fatty acids, which are invariably present in gram-negative bacteria as constituents of cell wall lipopolysaccharides, were absent. Furthermore, water-soluble lipopolysaccharides were not extracted from the cells with 45% aqueous phenol (7). The results of these studies indicated that despite having a negative Gram reaction, the cells might have a

cell wall structure of the type ordinarily associated with gram-positive bacteria. Therefore, cells of strain VPI 0310 were examined with an electron microscope and compared with cells of a strain of *Fusobacterium nucleatum*, which has a cell wall structure typical of gram-negative cells (2, 22). Also, by using light microscopy, we examined Gram-stained smears of strain VPI 0310 cultured for different periods of time and unstained preparations of living cells.

MATERIALS AND METHODS

Microorganisms. *F. plautii* strain VPI 0310 (= ATCC 29863 = Prévot S1) was obtained from the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg. *F. nucleatum* strain Fev1 was supplied by S. E. Mergenhagen, National Institute of Dental Research, Bethesda, Md.

Cultural conditions. Cells were cultivated at 37°C in narrow-necked, screw-capped bottles filled to the top with medium containing the following (in grams per liter): tryptone (Oxoid Ltd., London, England), 15.0; NaCl, 5.0; KH₂PO₄, 1.5; Na₂HPO₄·2H₂O, 3.5; NaHCO₃, 0.5; yeast extract (Oxoid), 3.0; L-cysteine hydrochloride, 1.0; vitamin B₁₂, 0.001; hemin, 0.005; menadione, 0.001; and glucose, 2.5. The pH of the medium was 7.0. Cells in the exponential or stationary phase of growth were harvested by centrifugation, washed once in phosphate-buffered saline (pH 7.2), and prepared for electron microscopy as described below.

For the study of cell morphology in unstained preparations and in Gram-stained smears, cells of strain VPI 0310 were cultivated in a modified peptone yeast extract glucose medium (8) containing tryptone instead of peptone, 0.08% agar, and ascorbic acid as a reducing agent. Narrow tubes filled with the semifluid medium were inoculated with cells from an exponentially growing culture and incubated for 3, 6, 12, or 24 h or for 2, 6, or 10 days.

Electron microscopy. Electron microscopy was carried out essentially as described by Kellenberger et al. (12); 3% glutaraldehyde in 0.2 M cacodylate buffer (pH

7.4) (18) was used as the fixative, and Spurr medium (21) was used for embedding. Embedded preparations were cut into thin sections (40 to 60 nm) with an ultramicrotome (LKB Produkter, Uppsala, Sweden), and the thin sections were transferred to grids (150 mesh). The grids were treated with 2% aqueous uranyl acetate for 30 min, thoroughly rinsed in water, treated with Reynolds lead citrate for 15 min, and finally washed in water.

Negative staining was performed with 2% aqueous phosphotungstic acid adjusted to pH 6.6.

All electron microscopic observations were made with a Hitachi model HU 12A electron microscope at 100 kV.

Gram staining. Gram staining was performed as outlined in the *Manual of Clinical Microbiology* (13); Gram crystal violet (Difco Laboratories, Detroit, Mich.) and ethyl alcohol (as decolorizer) were used.

Examination for motility. Hanging drop preparations of cells in the exponential phase of growth were examined by phase-contrast microscopy.

RESULTS

In Gram-stained smears, cells of *F. plautii* VPI 0310 appeared as straight or slightly curved rods 5 to 10 μm long with rounded ends. Some cells were fusiform. Cells grown for 48 h or less were uniformly gram negative. Gram-positive cells or gram-negative cells containing gram-positive granules were not observed. In addition, older cultures contained cells with bulbous swellings or vacuoles, as well as a few filaments. Structures resembling endospores were not observed. In unstained preparations the cells were nonmotile.

Cells of strain VPI 0310 appeared in thin sections as elongated structures whose shorter diameter ranged from 480 to 680 nm. In a few cells a membranous structure, interpreted as the plasma membrane, was partly discernible in close contact with the cytoplasm (Fig. 1a through c). The multilayered cell wall was approximately 50 nm wide. The dominating structure was a densely stained layer 5 nm wide in close contact with the plasma membrane or separated from it by an unstained or lightly stained space. This layer was present in cross walls between adjacent cells (Fig. 1a and b); most likely it represents peptidoglycan. All cells contained a thick outer layer (approximately 20 nm wide) that was slightly separated from the densely stained structure or in close contact with it. This layer followed a straight-line course and was quite distinct from the wavy, triple-layered outer membrane of *F. nucleatum* Fev1 (Fig. 1d). Also, the *F. nucleatum* cells had a clearly visible triple-layered plasma membrane.

The cytoplasm of both *F. plautii* and *F. nucleatum* consisted of a granular, electron-dense material, whereas the nuclear regions displayed a less dense, filamentous structure (Fig. 1b).

Membranous structures suggestive of mesosomes were not observed. Peculiar unstained rod-shaped areas were observed in some cells (Fig. 1a). Flagella were not observed in negatively stained preparations.

DISCUSSION

In thin sections, *F. plautii* VPI 0310 displayed a cell wall profile distinct from that of *F. nucleatum* Fev1, which showed a cell wall profile typical of gram-negative bacteria (3). Also, the cell wall of VPI 0310 differed from the rather thick and homogeneous wall of a typical gram-positive bacterium (4). Cell boundary layers more or less similar to those of *F. plautii* VPI 0310 have been observed in the sporeformers *Bacillus polymyxa* (16) and *Bacillus sphaericus* (10), in the nonsporeforming anaerobe *Eubacterium saburreum* (6), and in *Gardnerella vaginalis* (1, 5). These organisms have in common the ready loss of Gram positivity in aging cultures.

The lipopolysaccharide-lipoprotein complex of a gram-negative bacterium is associated with the outer, triple-layered membrane. The complete lack of such a membrane in *F. plautii* VPI 0310 explains why lipopolysaccharide could not be obtained from whole cells by extraction with 45% aqueous phenol (7) and why hydroxy fatty acids were absent in cell methanolysates (11).

The results of this study and of earlier investigations (7, 11) suggest that the strains of *F. plautii* are representatives of an anaerobic, nonmotile, nonsporeforming, gram-positive bacterium. This bacterium is certainly not identical to the oral organism (or organisms) described by Plaut (17) and Séguin (19). The drawings of Séguin (19) of vibrios and long bacterial cells with several curves are suggestive of a spirillum. We have observed similar morphological forms in Gram-stained smears from cultures of an oral strain of *Selenomonas*.

Two important taxonomic considerations have emerged from our studies. First, despite having a negative Gram reaction, *F. plautii* is a nonmotile, gram-positive organism. And second, the placement of this organism in the genus *Fusobacterium* is in error; the biochemical properties of the known strains of *F. plautii* (15), including the type strain, particularly the production in peptone yeast extract glucose broth of butyric acid as a major product together with smaller amounts of acetic, lactic, and succinic acids, fit the generic description of *Eubacterium* (9), but they are not fully compatible with the description of any of the *Eubacterium* species listed in *Bergey's Manual*, 8th ed. Therefore, we suggest a transfer of this species to the genus *Eubacterium*, as *Eubacterium plautii* (Séguin) comb. nov.

FIG. 1. Electron micrographs of ultrathin sections of strain VPI 0310 (a through c) and *F. nucleatum* strain Fev1 (d). PM, Plasma membrane; OM, outer membrane; OL, outer layer; PG, peptidoglycan; RS, rod-shaped structure; N, nuclear region. Bars = 200 nm.

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